

# A histomorphometric study of osteomalacia in elderly females with fracture of the proximal femur

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## SUMMARY

*Using standard histomorphometric indices on bone biopsies of trabecular bone volume, osteoid volume, the trabecular osteoid surface, the extent of the calcification front and the number of osteoid lamellae, a histomorphometric diagnosis of osteomalacia was made in three of 28 elderly female patients with fracture of the proximal femur. These patients also showed biochemical changes in the serum and deficiency of serum vitamin D. The 25 biopsies judged not to show osteomalacia showed a greater osteoid volume in the 12 patients who suffered an intertrochanteric fracture than in the 13 with a cervical fracture. Clinical biochemistry in these 25 patients showed considerable overlap between the normal range and that found in the patients with osteomalacic biopsies.*

## INTRODUCTION

Proximal femoral fracture is predominantly a problem of the elderly, affecting females over twice as commonly as males, and with an exponential rise in incidence with increasing age. This common problem in elderly females has been attributed in part to a reduced bone mass as a consequence of postmenopausal osteoporosis. Osteoporosis is defined as a condition in which the absolute amount of bone is diminished while the remaining bone is normal, whereas osteomalacia is characterised by deficient mineralisation of normal bone matrix.

The importance of osteomalacia as a preventable cause of fracture of the proximal femur has attracted considerable attention, particularly as treatment with vitamin D reverses the defective mineralisation of bone. However, the role of osteomalacia as a contributory factor to the aetiology of proximal femoral fracture remains in dispute. Histological evidence of osteomalacia has been reported in 20% of proximal femoral fracture patients from Scotland,<sup>1</sup> 30% from Wales,<sup>2</sup> 16-34% of females from Leeds<sup>3</sup> and 33% of females from London.<sup>4</sup> Other series of patients in Scotland and England have shown histological evidence in only 10% or less of fracture patients.<sup>5-7</sup> A large part of the variation between these studies can be attributed to the different histological criteria used to diagnose osteomalacia. In addition to differing methods of measurement, different parameters were employed which included osteoid volume,<sup>1, 2</sup> osteoid surface and mineralisation front,<sup>3</sup> and osteoid thickness.<sup>5, 6</sup> Nevertheless, there

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appears to be an increased prevalence of osteomalacia among elderly patients with proximal femoral fracture, which may predispose to the fracture. Such fractures appear likely to result either from the reduced strength of osteomalacic bone or from the myopathy associated with osteomalacia resulting in reduced mobility and an increased risk of falls.

## PATIENTS

Between August 1983 and July 1984, bone biopsies were obtained at the time of surgery for internal fixation of proximal femoral fracture from 28 female patients over age 65 years. The indications for biopsy were either as part of a research study approved by the ethical committee of treatment regimes for osteoporosis, or because osteomalacia was suspected on clinical or biochemical grounds. The former group (24 patients) were mentally alert and receiving few drugs and were considered unlikely to have osteomalacia, while the latter group (four patients) were considered likely to have osteomalacia.

## METHODS

Iliac crest biopsies were obtained in 26 subjects using a transiliac biopsy trephine, and in two subjects samples were obtained from the excised femoral head. The biopsies were prepared undecalcified, embedded in methyl methacrylate following the method of Difford<sup>8</sup> and sectioned using a motorised microtome. Sections were stained with aqueous toluidine blue which stains mineralised bone purple and osteoid blue, and also with an ethylene diamine tetra-acetic acid solution of toluidine blue which stains mineralised bone pink, osteoid light blue and the calcification front dark blue (Fig 1). The undecalcified sections obtained from the centre of the specimens were quantified using a planimetry system (MOP Videoplan Image Analysis System). The movable image of a liquid crystal diode was superimposed on the imaged field of the stained sections, enabling measurement to be carried out of the area of trabecular bone, area of osteoid, total length of trabecular surfaces and length covered by osteoid, and the proportion of osteoid surfaces with a calcification front. The maximum numbers of birefringent lamellae of osteoid were observed and counted at 100 × magnification under polarised light (Fig 2). The following histomorphometric indices were calculated from the measurements:

Trabecular bone volume (TBV) %	=	$\frac{\text{Area of trabecular bone (osteoid + mineralised)}}{\text{Total area (trabecular bone + inter-trabecular area)}} \times 100$
Relative osteoid volume (ROV) %	=	$\frac{\text{Area of osteoid}}{\text{Area of trabecular bone (osteoid + mineralised)}} \times 100$
Relative trabecular osteoid surface (ROS) %	=	$\frac{\text{Trabecular surface length covered by osteoid}}{\text{Total surface length of trabecular bone}} \times 100$
Extent of calcification front (CF) %	=	$\frac{\text{Surface length of osteoid with calcification front}}{\text{Surface length of osteoid}} \times 100$

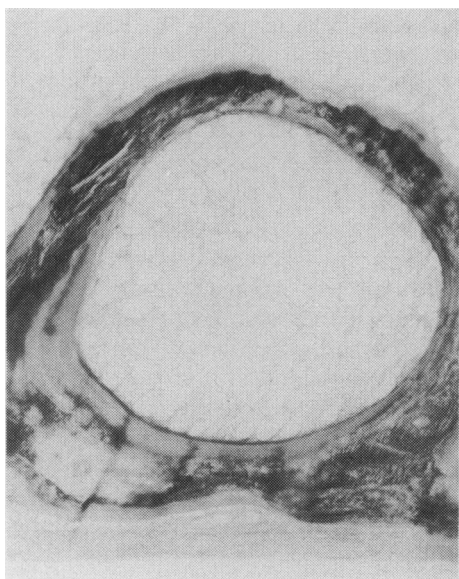
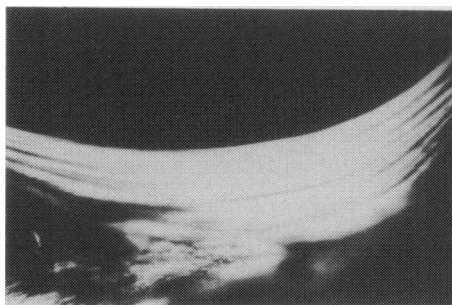


Fig 1 (left). Undecalcified section of iliac crest biopsy with trabecular surfaces covered by a thickened layer of osteoid indicative of osteomalacia (Case 1). Toluidine blue stain,  $\times 100$ .

Fig 2 (below). Osteoid seam with lamellar pattern viewed under polarised light (Case 3),  $\times 200$ .



A histological diagnosis of osteomalacia was considered if the relative osteoid volume was  $>3.5\%$ , the relative trabecular osteoid surface  $>24\%$ , the calcification front  $<60\%$ , or the maximum number of birefringent osteoid lamellae in excess of three.

A venous blood sample was withdrawn at the time of admission to estimate corrected serum calcium,<sup>9</sup> serum phosphate, alkaline phosphatase, albumin, globulin, urea, 25-hydroxyvitamin D (25 OHD) and 1,25 dihydroxyvitamin D (1,25 OH<sub>2</sub>D) levels. Fractures were divided into cervical and intertrochanteric (including basal cervical), according to radiographic appearances. Results were analysed using the Mann-Whitney U test, and mean and standard deviation calculated for comparisons between groups.

## RESULTS

Sixty-four female patients with proximal femoral fracture were admitted during the study period, and satisfactory histological analyses of undecalcified bone biopsies obtained in 28 of these. Their histomorphometric indices are shown in Table I and biochemical measurements in Table II. Three of these patients (11%) had histomorphometric evidence of osteomalacia. These three patients had already been suspected on the basis of a raised serum alkaline phosphatase in all three, low serum phosphate in two, and a low serum calcium in one. The two patients who had not received supplementary vitamin D had very low serum 25-hydroxyvitamin D and plasma 1,25 dihydroxyvitamin D. The mean values of these biochemical measurements in the two fracture subgroups judged not to have osteomalacia showed no significant differences. The corrected serum calcium was low in four of these patients, serum phosphate was low in one, serum alkaline phosphatase elevated in nine, and serum 25-hydroxyvitamin D was less than 25 nmol/l in 14 patients.

TABLE I

*Histomorphometric indices in three patients judged to have osteomalacia, and in 25 patients judged not to have osteomalacia. The patients without osteomalacia have been divided into a group of 13 with cervical fracture and a group of 12 with intertrochanteric fracture*

	Trabecular bone volume (%)	Relative osteoid volume (%)	Relative trabecular osteoid surface (%)	Calcification front (%)	Maximum number osteoid lamellae
OSTEOMALACIA					
Case 1	22	9.0	72	78	5
2	26	11.6	89	58	3
3	19	12.3	23	48	4
NO OSTEOMALACIA					
Cervical fracture (n = 13) (mean $\pm$ SD)	19.6 ( $\pm$ 6.6)	0.92* ( $\pm$ 0.6)	7.8 ( $\pm$ 7.6)	83.5 ( $\pm$ 4.6)	1.5 ( $\pm$ 0.7)
Intertrochanteric fracture (n = 12) (mean $\pm$ SD)	15.9 ( $\pm$ 6.6)	2.02* ( $\pm$ 1.1)	10.7 ( $\pm$ 6.8)	79.3 ( $\pm$ 9.1)	1.4 ( $\pm$ 0.5)

\*Significant difference  $p = 0.01$ .

TABLE II

*Biochemical measurements in the three patients judged to have osteomalacia and in the two groups of patients judged not to have osteomalacia*

	Corrected serum calcium (mmol/l)	Serum phosphate (mmol/l)	Serum alkaline phosphatase (IU/l)	Serum urea (mmol/l)	Serum 25-OH D (nmol/l)	Plasma 1,25 OH <sub>2</sub> D (pmol/l)
OSTEOMALACIA						
Case 1	2.22	0.88	423	12.3	9	<11
2	2.21	0.63	243	10.3	71*	49*
3	2.11	0.54	394	9.7	4.5	<10
NO OSTEOMALACIA						
Cervical fracture (n = 13) (mean $\pm$ SD)	2.28 ( $\pm$ 0.1)	1.03 ( $\pm$ 0.2)	175.1 ( $\pm$ 50)	8.8 ( $\pm$ 3.7)	23.7 ( $\pm$ 18)	17.4 ( $\pm$ 11.1)
Intertrochanteric fracture (n = 12) (mean $\pm$ SD)	2.30 ( $\pm$ 0.1)	1.02 ( $\pm$ 0.1)	179.2 ( $\pm$ 36)	8.2 ( $\pm$ 3.1)	26.1 ( $\pm$ 12)	18.2 ( $\pm$ 7.6)

\*Vitamin D therapy had been commenced three weeks before the proximal femoral fracture.

The relationship between corrected serum calcium and serum 25-hydroxyvitamin D is shown in Fig 3. Neither of these measurements identified all three of the osteomalacic patients. A considerable number of the patients not judged to be osteomalacic have low values of one or the other, or both, measurements.

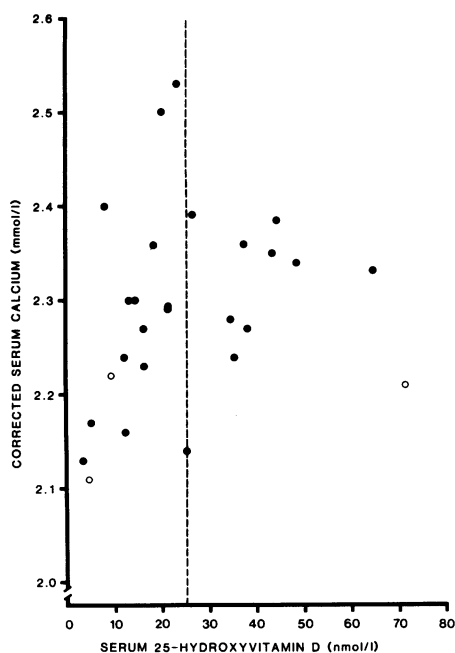


Fig 3. Serum calcium and 25-hydroxy-vitamin D (normal > 25 nmol/l) in elderly females with proximal femoral fracture with (o) and without (●) histomorphometric evidence of osteomalacia.

The biopsies in 25 patients showed no histomorphometric evidence of osteomalacia. One patient with radiological evidence of Paget's disease of bone involving the pelvis had accompanying histological evidence with increased woven bone within the trabeculae compatible with this diagnosis. These 25 patients have been subdivided into 13 with a cervical fracture (mean age 80 years) and 12 with an intertrochanteric fracture (mean age 81 years). The only significant difference between these two groups was that the relative osteoid volume was greater for those with an intertrochanteric fracture, although none reached the level necessary for classification of osteomalacia.

## DISCUSSION

Osteomalacia was confirmed in three of 28 (11%) elderly female patients with proximal femoral fractures selected for this study. Selection was conducted neither randomly nor in a controlled prospective fashion and these patients may therefore not fully represent the population from which they were drawn.

Those with low mental test scores, who are more frail and have an increased likelihood of vitamin D deficiency, were excluded from the research study group (24 patients), while the other four patients were considered likely to have osteomalacia. The prevalence of osteomalacia was similar to the 10% reported in a study of patients with proximal femoral fracture in Glasgow,<sup>7</sup> in which similar histological diagnostic criteria were used, and where latitude and hours of sunlight are comparable with those in Belfast.

The biochemical results emphasise that corrected serum calcium measurements may be normal in patients with osteomalacia.<sup>10</sup> Similarly, serum phosphate may be unhelpful<sup>11</sup> although hypophosphataemia often precedes the development of hypocalcaemia.<sup>12</sup> The serum alkaline phosphatase was markedly elevated in the patients with osteomalacia, and, although this abnormality is not specific, the elevation as a consequence of fracture alone was usually less than twice the upper limit of normal (340 IU/l) and indeed was not elevated in 16 cases (57%) at the time of admission. The two patients with untreated osteomalacia had serum 25-hydroxyvitamin D levels of less than 25 nmol/l (10 ng/ml), the level below which osteomalacia is considered to occur.<sup>13</sup> However, 14 patients had markedly lowered serum 25-hydroxyvitamin D values but without histomorphometric changes of osteomalacia, which confirms that low values in the elderly may occur in the absence of osteomalacia.<sup>14</sup>

Increased trabecular bone volume<sup>15, 16</sup> and reduced amount of osteoid<sup>17</sup> in patients with cervical fractures in comparison with trochanteric fractures have

been noted previously. In this series there was no significant difference in mean serum 25-hydroxyvitamin D levels between the two fracture groups, although lower 25-hydroxyvitamin D values<sup>13</sup> and a higher incidence of osteomalacia have been reported in trochanteric fractures.<sup>18</sup> While the increased amount of osteoid in patients with trochanteric fractures may occur as a consequence of deficiency of vitamin D and a mild mineralisation defect, the poorer physical status and accompanying disease processes in this type of fracture may be of equal importance, causing increased bone turnover and a resultant overall increase in bone resorption.

The incidence of osteomalacia amongst elderly hospital admissions has been shown to be highest in females and those over 70 years of age.<sup>19</sup> This same population also carries the highest risk of sustaining a fracture of the proximal femur, and thus the two conditions may occur together without the relationship being causal. Minor increases in osteoid have been associated with an increased risk of fractures of the proximal femur,<sup>3, 17, 20</sup> and bone strength has been shown to be significantly related to the amount of osteoid.<sup>1</sup> Careful screening of fracture patients to identify this preventable factor must be important, since even at this late stage treatment with calciferol, which is simple, cheap and effective, will aid fracture healing<sup>11</sup> and prevent further fractures. Additionally, the resolution of musculoskeletal symptoms accompanying osteomalacia is of considerable symptomatic benefit to the patient with consequent improvement of mobility and independence.

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